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(56) Documents Cited

GB 2018600 A

GB 1442419 A

GB 1294557 A

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(58) Field of Search

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(54) **Cleaning method and formulation**

(57) A method for cleaning a surface carrying proteinaceous material, which comprises applying to the surface one or more enzymes that digest the material, and then wiping the surface.

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CLEANING METHOD AND FORMULATION

This invention relates to a cleaning method, and in particular to an enzyme formulation.

Cleaning materials used at present rely on very strong
5 solvents to remove dirt present in surfaces and often still
require a low of physical exertion to produce the required
results. These cleaners, containing high concentrations of
solvents and other harmful chemicals such as ammonia, are
harmful to the environment, create a dangerous health and
10 safety situation (they are often inflammable as well as
toxic), and can damage surfaces they are applied to,
especially paintwork.

Dirt on surfaces often contains a biological component
which often acts like a "glue" which binds together soil
15 particles and makes the dirt harder to remove. A
particular example is of squashed flies or other insects
that accumulate on car windscreens and paintwork, which
smear over the surface and bind dust and debris to it.

The present invention is based on the realisation
20 that, in order to solve the problems described above,
biological catalysts can be used to speed up the reactions
that naturally occur between the chemicals present in the
environment or supplied, and the matter on the surface to
be cleaned, thereby breaking down, or "digesting" the
25 matter. This will enable the material to be removed from
the surface more easily.

A formulation for use in the invention will usually
contain a number of enzymes; the exact types used and their
relative concentrations can be modified to tailor the
30 formulation to the exact purpose to which it is to be put.
This can be related to the "average" composition of the
biological material on the surfaces. In the case of the
windscreens or paintwork covered in squashed flies and road
debris, enzymes such as chitinases, proteases and
35 cellulases prove effective in "cutting up" the "glue" and
making the surface easy to clean.

The inclusion of proteases allows the enzymes (which are proteins themselves) to be degraded and so to reduce the activity of the enzymes with time. The formulation is thus "environmentally friendly". It has been found that
5 this action is relatively slow, at least insofar as it is desirable to ensure that the non-protease activity is not compromised in use of the formulation.

The formulation will usually be used in aqueous or other liquid form. No surfactant is necessary. For
10 storage, the components may be freeze-dried.

A liquid formulation can be delivered to the surface to be cleaned by means of a pump action spray. Alternatively, it may be used in the car wash bottle, enabling easy application to the windscreen and headlights.

15 The enzymes may be free in solution, or they may be on some support, such as kaolin, kieselguhr or diatomite earth. This has the added bonus of having a mild abrasive effect, allowing even more effective cleaning of the surfaces.

20 The solution of the enzymes can be either aqueous, or contain more powerful solvents to enable an additional "dry cleaning" effect on the surface, by dissolving away the materials present.

Proteases suitable for use in the invention are
25 widely-available. Sources include Sigma, SAF, Boehringer and Biocatalysts Ltd. They may be obtained from various organisms, including mammals, bacteria and fungi.

Insect material (from flies, gnats, moths, butterflies, etc) is composed of proteinaceous material,
30 liquid, cellulose, pectin, hemicellulose, xylans, chitins and a variety of carbohydrates. Enzymes for use in the invention may be chosen accordingly, although not all substrates necessarily have to be digested, in order to utilise the invention.

35 The following Examples illustrate the invention.

Example 1

General degradation of insect material was assessed using a crude digestion assay system comprising of insect material (whole or crushed, wet and dried samples) of 150-
5 750 mg wet weight in 0.5 ml buffer (phosphate or Tris 50-300 mM at pH 6.5-7.5, preferably 7.0), all incubated at 25-37°C, preferably 30°C. To the substrate mixture, 0.2 ml of biocatalyst suspension was added: suspensions of commercially-available enzymes were used neat (when in
10 liquid) or resuspended freeze-dried samples in phosphate or Tris buffers as above. Reactions were incubated for 20 minutes but not stirred or shaken. The production of general amino-acid content was measured against a suitable background control, using TLC, MPLC, Folin & Ciocalteu's
15 Reagent, Brilliant Blue G or other standard amino-acid and peptide assays known to those skilled in the art. Proteases from Bacillus, Aspergillus, Rhizopus and Streptomyces were particularly effective at releasing large quantities of amino-acids within 5-10 minutes of
20 incubation.

Experiments using different combinations of protease mixtures showed an "additive" effect, particularly with combinations of bacterial and fungal enzymes. This indicated different cleavage mechanisms and kinetics of the
25 individual component enzymes of each formulation; the resultant effect was concomitant release of substantially more amino-acids than with either component enzyme alone, even after prolonged incubation at 37°C.

These results indicated that commercial protease
30 formulations (crude preps) were individually and collectively useful at degrading some of the substructure of insect material, resulting in a loosening of the cellulose-based fabric and exoskeleton and enabling greater accessibility for further breakdown by other enzyme
35 preparations.

Example 2

Using the same experimental procedures as in Example 1 and assay techniques familiar to those skilled in the art, insect substrates were exposed to a wide variety of
5 amylases, glucanases, xylanases, hemicellulases, cellulases, pectinases and chitinases. The corresponding release of sugar products was followed using standard sugar assay techniques. All enzyme preparations tested were able to release considerable quantities of saccharides,
10 disaccharides and polysaccharides from ground insect material.

Example 3

Combinations of cellulases from *Aspergillus* and *Trichosporon*, proteases from *Streptomyces*, *Aspergillus*,
15 *Rhizopus*, amylases from *Bacillus* and *Aspergillus*, and lipases from *Candida*, *Mucor* and *Rhizopus*, were tested in a variety of different concentrations upon insect material (100 mg in 3 ml 50 mM buffer, pH 7.0, at 25°C). Release of sugars, amino-acids, fatty acids, etc was monitored using
20 HPLC and shown to occur simultaneously over an initial period (3-7 minutes), but decreased after 15 minutes, probably due to cannibalism of the other lytic enzymes by the powerful protease content.

These experiments indicated that a variety of enzyme
25 activities could perform to the same degree in the presence of each other's activities with release of water-soluble monounits and concomitant collapse of the insect structure.

Example 4

Bottles of enzyme formulations were prepared using 5%
30 total enzyme concentrations with varying mixtures of proteases, lipases, cellulases and amylases from 0-100% of the enzyme content of each formulation. In addition, abrasive agents such as vermiculite, kieselguhr and Fuller's earth were added in finely ground particulate form
35 and ethanol (0.5-2%) was mixed into the final formulation.

Windscreens with dried insect debris were prepared, throughout June and July, by driving through country lanes,

and not using the windscreen wash prior to the experiments. 500 ml bottles of each formulation were prepared, shaken vigorously before use and sprayed (using a standard pump and nozzle bottle) over one half of the windscreen. The other half was protected by newspaper to prevent carry-over. Each treated area was left for 10 minutes before applying the standard windscreen wash and wipers (water and detergent) with 10 swipes of the wipers. All of the formulations had some effect, better than the use of water or buffer alone.

The most marked removal of insect debris was achieved using formulations containing 40-60% protease, 10-30% lipase, 20-40% cellulase and 10-20% amylase. The optimum formulation appeared to be 40% protease (fungal and bacterial mix), 10% lipase, 35% cellulase and 15% amylase.

The presence of vermiculite or Fuller's earth particles improved the clearance of debris from the windscreen, as did ethanol at up to 20%.

CLAIMS

1. A method for cleaning a surface carrying proteinaceous material, which comprises applying to the surface one or more enzymes that digest the material, and then wiping the surface.
2. A method according to claim 1, wherein the surface is an automobile windscreen.
3. A method according to claim 1 or claim 2, wherein the one or more enzymes are applied in a liquid film over the surface, by operation of an applicator containing the liquid.
4. A method according to claims 2 and 3, wherein the one or more enzymes are applied as a component of the windscreen wash liquid.
5. A method according to any preceding claim, wherein the one or more enzymes are selected from chitinases, cellulases and proteases.
6. A method according to any preceding claim, wherein a clay-like particulate material is also applied.
7. A method according to any preceding claim, wherein the enzymes are applied in an aqueous system comprising ethanol.
8. A method according to claim 1, substantially as exemplified herein.
9. A formulation comprising the enzymes defined in claim 5.
10. A formulation according to claim 9, additionally comprising a clay-like particulate material.
11. A formulation comprising protease and a clay-like particulate material.
12. A formulation according to claim 11, additionally comprising chitinase and/or cellulase.
13. A formulation according to any of claims 9 to 12, in an aqueous medium comprising ethanol.
14. A formulation according to any of claims 9 to 13, comprising bacterial and fungal proteases.

Patents Act 1977
Examiner's report to the Comptroller under Section 17
(The Search report)

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Relevant Technical Fields

- (i) UK Cl (Ed.) CSD DGA
(ii) Int Cl (Ed.)

Search Examiner
M ELLIOTT

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Databases (see below)

- (i) UK Patent Office collections of GB, EP, WO and US patent specifications.

Documents considered relevant following a search in respect of Claims :-
1-14

(ii)

Categories of documents

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|---|---|
| X: Document indicating lack of novelty or of inventive step. | P: Document published on or after the declared priority date but before the filing date of the present application. |
| Y: Document indicating lack of inventive step if combined with one or more other documents of the same category. | E: Patent document published on or after, but with priority date earlier than, the filing date of the present application. |
| A: Document indicating technological background and/or state of the art. | &: Member of the same patent family; corresponding document. |

Category	Identity of document and relevant passages	Relevant to claim(s)
X	EP 0181651 A2 (UNILEV) whole document	1 at least
X	EP 0125801 A1 (GENEX CORPORATION) whole document	1 at least
X	GB 2019600 A (SENJU SEIYAKUKK)	1 at least
X	GB 1442419 A (PROCTER & GAMBLE CO)	1 at least
X	GB 1294557 A (FUJI PHOTO FILM CO)	1 at least
X	GB 1277479 A (PROCTER & GAMBLE CO)	1 at least

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